Pressor Polypeptides Formed in Vivo and in Vitro as Mediators of Renal Hypertension

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During the purification of hypertensin formed in vitro, evidence of heterogeneity of the pressor activity was obtained by counter current distribution. Pepsitensin shows a similar heterogeneity. These facts lead to the hypothesis that other proteolytic enzymes may also be present in the tissues and may hydrolyze hypertensinogen with the formation of pressor polypeptides of the hypertensin or pepsitensin type. Thus, substances similar to hypertensin may be formed in the organism by the action of proteolytic enzymes other than renin and may, if produced locally, exert a vasoconstrictor action or, if they enter the circulation, cause an increase in blood pressure.

THE importance of chemical mediators in normal and pathologic conditions is now universally recognized. The term "local hormones" was introduced not long ago by Professor Gaddum and is applied to "pharmacologically active substances [having] important local functions in the regulation of tissue activity, particularly of involuntary muscles, gland cells and capillaries."[1] This term does not include those substances which are set free in normal or injured tissue and may act on distant organs. Many of these occur naturally and may be considered as true hormones, while others have been proved to be formed only under artificial conditions and their function in physiologic or pathologic conditions is thus only hypothetical.

The polypeptides form an important group of these substances. Recently pharmacologically active organic acids have been shown to be formed in vitro and in vivo[2] and some of them have vasoconstrictor and pressor activities.[3, 4] Table 1 gives a list of some pharmacologically active polypeptides. All of those formed in vitro by the action of proteolytic enzymes have the same substrate (α-globulin). Only vasopressin, hypertensin, and pepsitensin have a pressor action when injected intravenously.

Vasopressin has been purified, its composition and amino acid sequence recognized, and its synthesis achieved. We know, nevertheless, that there are at least 2 vasopressins according to their origin (hog or bovine) and differing only by the presence or absence of 1 amino acid.[5]

Hypertensin, or more correctly, some of the hypertensins, have been purified and 2 of them synthesized.[6-10] During the purification of hypertensin prepared with hog renin and ox hypertensinogen, evidence of heterogeneity of the pressor activity was obtained by countercurrent distribution in the systems 2-butanol, 0.1 M ammonium hydroxide and 2-butanol, 0.05 M sodium phosphate buffer pH 7.65. These results led to the study of the countercurrent behavior of hypertensin preparations obtained with hypertensinogen from different animal species and hog renin. The results (fig. 1) show that hog, horse, and ox hypertensinogens each give rise to several active components on incubation with hog renin.[11] A similar heterogeneity was found in the hypertensin formed in the plasma of a dog after total renal ischemia of 6 hours' duration. The plasma was incubated at 2 C. for 24 hours. With some reservations it may be tentatively concluded that circulating hypertensin is also heterogeneous.[12]

At present it is difficult to explain the origin and physiologic importance of the hypertensins reported here, but it seems fairly well established that the preparations currently employed as starting materials for the purification may consist of complex mixtures. Attempts to distinguish some of the separate components pharmacologically have been unsuccessful up to now. Neither the pretreatment of the Nembutalized rat by different

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TABLE 1.—*Pharmacologically Active Polypeptides*

Active polypeptides	Inactivated by							Substrate destroyed by			
	Chymotrypsin	Trypsin	Pepsin	Action on B.P.	Other actions	Enzymes	Substrate	Previous renin	Heat	Alcohol ppt.	pH 3.9
Vasopressin	+	+	0	+	anti-diuretic	—	—				
Pepsanurin	+	+	0	0	anti-diuretic	pepsin	alpha-globulin	no	?	no	no
Oxytocin	+	0	0	−	uterus+		—				
Pepsitocin	+	0	0	0	uterus+	pepsin	alpha-globulin	no	?	no	no
Substance P	+	+	+	−	gut +						
Substance U	+	0	?	−	gut & uterus+	urine?	alpha-globulin				
Kallidin	+	0	?	−	gut & uterus+	kallikrein	alpha-globulin	?	no	?	yes
Bradykinin	+	0	?	−	gut & uterus+	trypsin	alpha-globulin	no	no	no	no
Hypertensin	+	+	+	+	gut & uterus+	renin	alpha-globulin	yes	yes	yes	no
Pepsitensin	+	+	+	+	gut & uterus+	pepsin	alpha-globulin	±	no	?	no

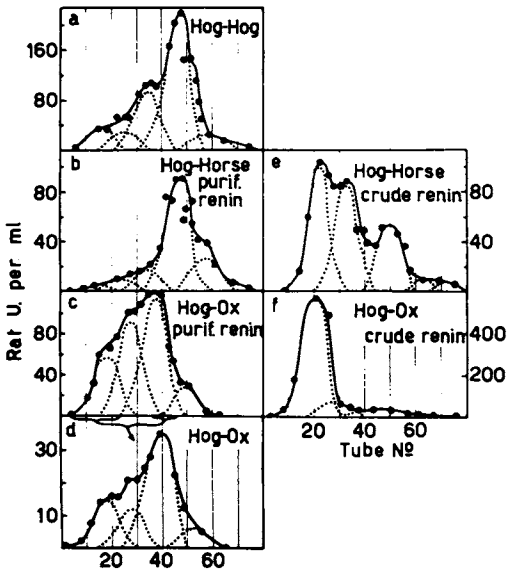

FIG. 1. Counter current distribution of different preparations of hypertensin. *Solid line,* experimental curve; *dotted lines,* theoretical distribution of a single substance; *Hog-Hog,* hog renin and hog hypertensinogen; *Hog-Horse,* hog renin and horse hypertensinogen; and so on. The pressor activity was measured in nephrectomized rats anesthetized with Nembutal. One rat unit is the pressor activity of 0.2 ml. of a standard hypertensin preparation which raises the blood pressure 20 to 35 mm. Hg and is equivalent approximately to 0.01 Goldblatt units. *(Reprinted from Biochem. et Biophys. Acta* 18: *580, 1955.)*

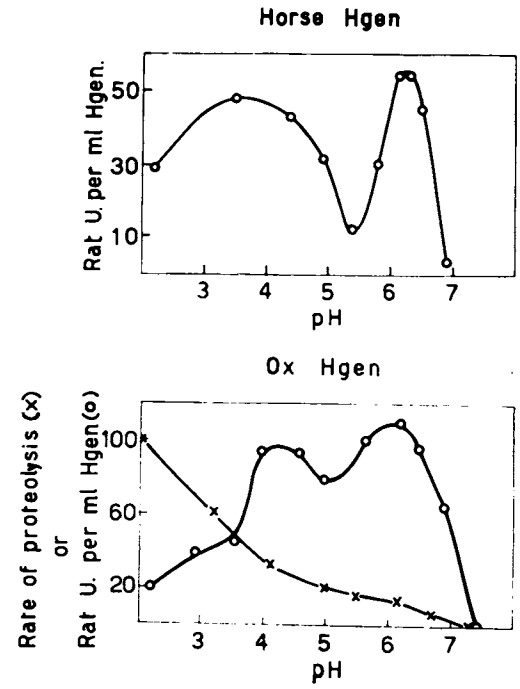

FIG. 2. Maximal production of pepsitensin in relation to pH.

drugs, nor parallel assays of vasoconstrictor and plain muscle stimulating action, in order to find a significant "index of discrimination," have given definite results.[13]

Pepsitensin is the product of hydrolysis of hypertensinogen (α-2-globulin) by pepsin. If the mixture of hypertensinogen and an adequate amount of pepsin is incubated a certain time, acidified to pH 5 with dilute HCl, and then brought to boiling in a water bath, a maximum production of pepsitensin is obtained independently of the time and temperature of incubation. Concentration of the enzyme and pH of the mixture, on the other hand are variables which determine the amount of pepsitensin formed. It was found that 3 to 5 mg. of crystallized pepsin per ml.

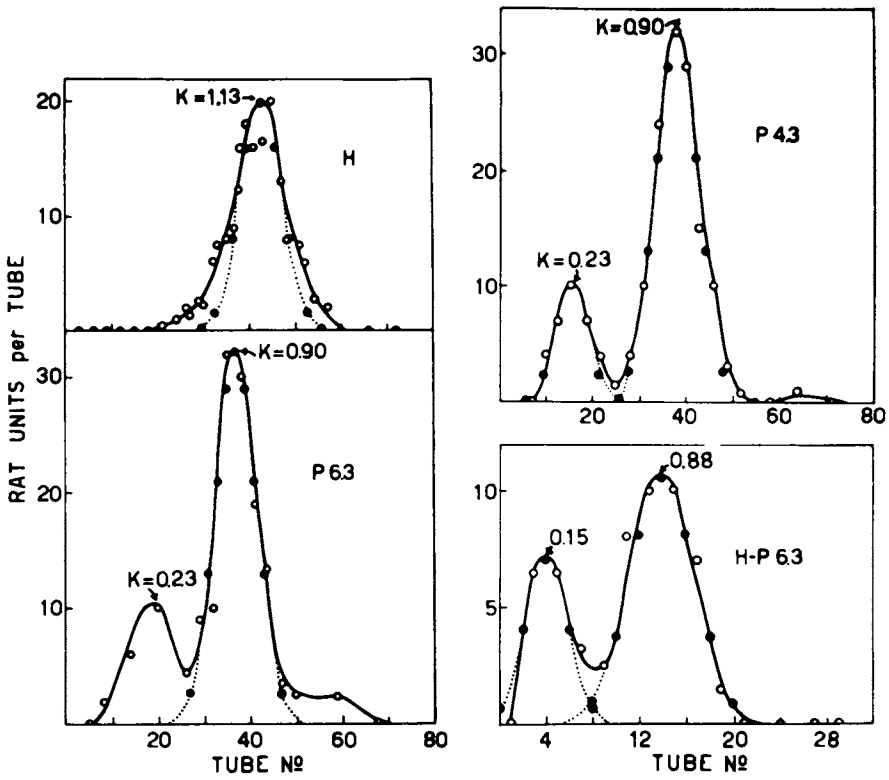

FIG. 3. Counter current distribution of bovine hypertensin *H* as compared with bovine pepsitensin obtained at pH 6.3 and 4.3 (*P6.3, P4.3*) and with pepsitensin obtained by the action of pepsin at pH 6.3 on heat coagulated bovine hyper-tensinogen previously treated with renin to produce maximal amounts of hypertensin (*H-P6.3*). Distribution of H-P6.3 was only of 3° equilibrations due to the small amount of material available.

TABLE 2.—*Maximal Production of Hypertensin and Pepsitensin*

Renin mg./ml. Hgen* pH 7.5	Rat U. per ml. Hgen	Pepsin mg./ml. Hgen pH 4.3	Rat U. per ml. Hgen	Pepsin mg./ml. Hgen pH 6.3	Rat U. per ml. Hgen
0.1	104	3.0	100	3.0	100
0.3	92	3.0	94	4.5	93
0.5	104	—	—	6.0	120
1.0	112	—	—	—	—
Av. 103		97		104	

*Bovine hypertensinogen: 1 ml. equivalent to 3 ml. of original plasma. Time of incubation at 30 C. for renin, 20 min. and for pepsin 5 or more min. Results are expressed in "rat units" of pressor activity, approximately equivalent to .01 dog (Goldblatt) units. Renin solution used contained 1 dog unit per ml.

of hypertensinogen is the optimum concentration (1 ml. of hypertensinogen is equivalent to 3 ml. plasma). At this concentration, maximal production of pepsitensin changes with pH of the mixture, showing maxima at approximately pH 4 and at pH 6.3.[13] As was known, the proteolytic activity measured by a modified Anson's method diminished with increasing pH (fig. 2).

A given amount of hypertensinogen yields equivalent pressor units of hypertensin or pepsitensin when treated with renin or pepsin under optimal conditions (table 2). Pepsin is able to form nearly maximal amounts of pepsitensin (80 per cent) when acting upon heat-coagulated hypertensinogen, and about 15 per cent of the normal production with heat-coagulated hypertensinogen previously treated with renin. Evidence of heterogeneity of the pressor activity of pepsitensin preparations was also obtained by counter current distribution (fig. 3).

The fact that a maximum of pepsitensin formation was observed at pH 6.3 led to the hypothesis that other proteolytic enzymes present in the tissues might hydrolyze hyper-

tensinogen with the formation of pressor polypeptides of the hypertensin or pepsitensin type. Extracts of spleen were prepared according to the method of Fruton and Bergmann[14] for cathepsin, and incubated with hypertensinogen. Experiments are still in progress, but in spite of some encouraging preliminary results, nothing definite can be stated regarding the production of pressor polypeptides by this reaction.

Recently Dengler[15] has presented evidence that the arterial wall contains a thermolabile, nondialyzable factor precipitated with ammonium sulphate between 0.3 and 0.6 saturation which, when incubated with plasma globulin, yields a substance with vasoconstrictor and pressor activity and which contracts the rat's uterus and the guinea pig's ileum. It could be argued that the arterial wall could contain renin, as suggested by Introzzi et al., [16] but apparently the arterial wall extract is not identical with renin. No definite proof is yet available of the polypeptide nature of the product of its reaction with plasma globulins; nevertheless this possibility must be borne in mind.

It does not seem illogical to assume that the production of pharmacologically active substances by the action of tissue enzymes on blood substrates may be one of the homeostatic mechanisms of the body. The formation of polypeptides by the action of proteolytic enzymes on blood globulin (especially α-2-globulin) is one of such mechanisms. Many polypeptides have been studied and there may be many more. The physiologic significance of bradykinin or bradykinin-like substances has recently been recognized as playing a role in changes of capillary permeability, production of local pain, and vasodilatation.[17] The facts discussed in this paper suggest that substances similar to hypertensin may be formed by the action of proteolytic enzymes other than renin and may, if produced locally, exert a vasoconstrictor action or, if they enter the circulation, produce an increase in blood pressure.

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tasks (*3*), regardless of whether the injury was in the right or left hemisphere, or in the frontal, parietal, temporal, or occipital lobes. The deficit on the Army General Classification Test turned out to be comparatively focal (*1*), and so were a number of other changes in performance, such as difficulty with route finding (*4*), which was limited to the group with parietal penetration.

The work of Lashley (*5*) and others on subhuman mammals can be similarly interpreted. For certain complex tasks, such as the maze, Lashley found general (nonlocalized) effects of cortical removals in rats. With other tests, in the same animals, he found focal changes such as alterations in brightness habit after occipital removals, and difficulties on a "double platform box" after anterior removals. Thus, specific and general effects coexist after cerebral lesions in man, as well as in subhuman forms; which of these effects appears to predominate depends on the nature of the tests employed. If the range of the tasks is sufficiently extended, one finds specific and general effects in obligatory association.

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1 November 1957

Thermal Decomposition of 8-Quinolinol Chelates of Silver (I)

The reaction of 8-quinolinol (oxine) with silver (I) has been the subject of a number of investigations. Vis (*1*) found that a green crystalline mass corresponding to the formula, $AgC_9H_6NO \cdot C_9H_6NOH$, was obtained from concentrated solutions of the reactants. Fox (*2*), however, obtained a green precipitate which corresponded to the 1:1 chelate, AgC_9H_6NO. From ionic charge considerations, one would expect the 1:1 chelate to be formed since the 8-quinolinol ion forms a five-membered ring containing one ionic and one covalent point of attachment to the metal ion.

The work of Tzinberg (*3*), which was confirmed by Hein and Regler (*4*), revealed that the formula advocated by Vis was correct. Further confirmation came from Block, Bailar, and Pearce (*5*), who were able to prepare two modifications of the chelate, a yellow form and a green one.

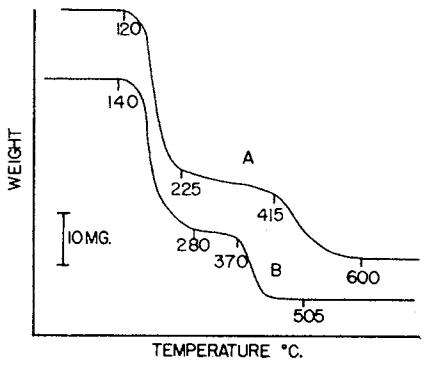

Fig. 1. Thermal decomposition curves of the 8-quinolinol chelates of silver (I). *A*, Green form; *B*, yellow form. Heating rate, 5.4°C per minute.

The nature of the bonding in the metal chelates is still open to question. It was first thought that silver (I) was oxidized to silver (II) and thus would have the normal bonding found in most of the metal 8-quinolinol chelates. If this were the case, the metal chelates would be paramagnetic; however, it was found that they were diamagnetic and hence, that they were silver (I) chelates (*5*). In view of these findings, it was suggested that the silver chelates were addition compounds containing an extra molecule of 8-quinolinol per molecule of silver chelate. This type of chelate, called a lattice chelate because the extra molecule of 8-quinolinol is thought to be held by weak lattice forces, is known for the 8-quinolinol chelates of Sc, Th, U (VI), and Pu (VI).

Now if the silver 8-quinolinol chelate contains solvated 8-quinolinol, it may be possible to prepare the normal 1:1 chelate by thermal decomposition. To find out whether this is possible, the two modifications of the silver 8-quinolinol chelate were prepared as previously described (*5*) and subjected to thermal decomposition on a thermobalance (*6*).

The thermal decomposition curves for the yellow and green forms of the silver 8-quinolinol chelate are given in Fig. 1. The yellow form was the more stable of the two modifications. It was stable up to 140°C when it began slowly to lose weight. The weight loss then became quite rapid, giving a break in the curve at 280°C; however, a constant weight level having the stoichiometry of the 1:1 chelate was not obtained. Beyond 370°C, further rapid weight loss took place to give the metallic silver level beginning at 505°C.

The green form began to lose weight at 120°C, giving a break in the curve at 225°C. Again, a horizontal weight level was not found for the 1:1 chelate. Beyond 415°C, further rapid weight loss took place to give the metallic silver level beginning at 600°C.

The results of these curves reveal that it is not possible to remove thermally the extra solvate molecule of 8-quinolinol without total disruption of the silver chelate. Such behavior is contrary to the thermal decomposition of the 8-quinolinol chelates of thorium and uranium (VI) but similar to that of scandium (*7*).

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14 October 1957

Suggested Revision of Nomenclature—Angiotensin

Concurrent discovery has become commonplace, almost as though a mental sputnik regularly circled the earth, distributing with abandon our most exciting thoughts. The vasoactive peptide resulting from the action of renin on an alpha-globulin was thus discovered by two groups of investigators with the result that the peptide received two trivial names, angiotonin and hypertensin. Synthesis of the octapeptide has now brought a degree of certainty about the identity of this peptide and justifies dropping the double nomenclature. We propose the simplified name, *angiotensin*, and its derivatives *angiotensinase* and *angiotensinogen*. *Angiotensin* is a hybrid word but does, we think, have the advantage of being easy to pronounce even with a variety of accents, and it is euphonious and is understandable despite the most recalcitrant microphone.

There will be many who from habit will want no change, but we hope usage will make the heart grow fonder.

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5 December 1957